Statement of the Swiss Expert Committtee for Biosafety on the classification of work with genetically modified viral vectors

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Lentivirus vectors

Replication defective Lentivirus vectors, such as the ones derived from the system of Didier Trono (for example: Nature Biotech. 15:871-875, 1997; J.Virol. 72:8463-8471, 1999; J.Virol. 73:2886-2892, 1999) are classified generally in group 2, if the inserted sequence does not belong to a higher group.

Example of a lentivirus vector:

- **Packaging construct:**
  CMV-promoter, drives expression of the viral proteins gag, pro, pol, required in trans. nef, vpr, vif, vpu and env are deleted.
- **Envelope construct:** encodes the VSV-G-protein for pseudotyping the particles.
- **Vector construct:** HIV cis-acting sequences required for packaging, reverse transcription and integration as well as cloning site for heterologous DNA.

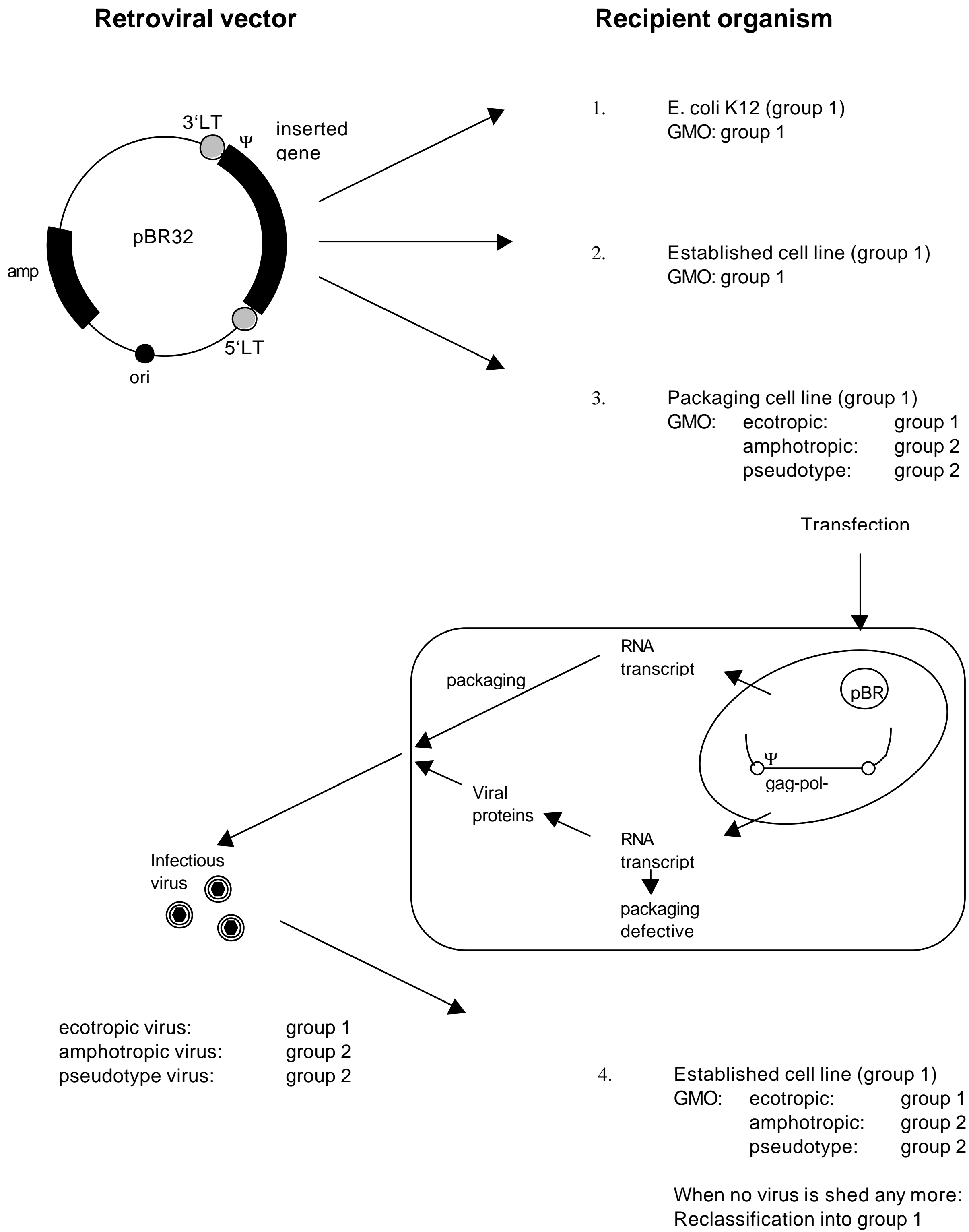
Retrovirus vectors

Retrovirus vectors are usually constructed as shuttle vectors and generally contain the plasmid (pBR322)- based sequences necessary for replication and selection in bacteria, the viral cis sequences necessary for integration, replication and RNA synthesis. The gene to be expressed is under the control of the strong LTR promoter. The viral gag, pol and env genes are deleted (see Fig. 1).

By transfection of retrovirus vectors into packaging cell lines, recombinant replication-defective virus (ecotropic virus: group 1; amphotropic virus: group 2; pseudotype virus: group 2) is produced; at the same time, replication competent retroviruses can be produced. Consequently, infection of cell lines with recombinant group 2 retroviruses is considered as a class 2 activity. The established cell line can only be classified under group 1 after it has been demonstrated that no replication competent viruses are produced any more.

For more detailed information on these vectors, see also the comments by the German “Zentrale Kommission für Biologische Sicherheit”, http://www.rki.de/GENTEC/ZKBS/ZKBS.HTM.

Retroviral expression



Amended: 5/01

Figure 1

Semliki forest virus (SFV) and Sindbis virus expression system

Semliki forest virus and Sindbis virus expression system consist of 2 components, the expression vector and the helper plasmid (see Fig. 2).
The expression of heterologous proteins in eucaryotic cells occurs after the transfection with RNA-transcripts derived from the linearised expression vector (Fig. 2a), or after infection with recombinant SFV- or Sindbis viruses produced by cotransfection of cultured cells with the expression vector and the helper plasmid (Fig.2b).

Expression vectors derived from Semliki forest virus are called pSFV1, 2 or 3; expression vectors derived from Sindbis virus are called pSinRep5. The expression vectors carry the cDNA of a defective SFV- or Sindbis virus genome, respectively, under control of the bacteriophage SP6-promotor. The region coding for the structural genes are deleted, but the cDNA carries the packaging signal (Vps), the complete non-structural genes and the subgenomic promoter (PSG).

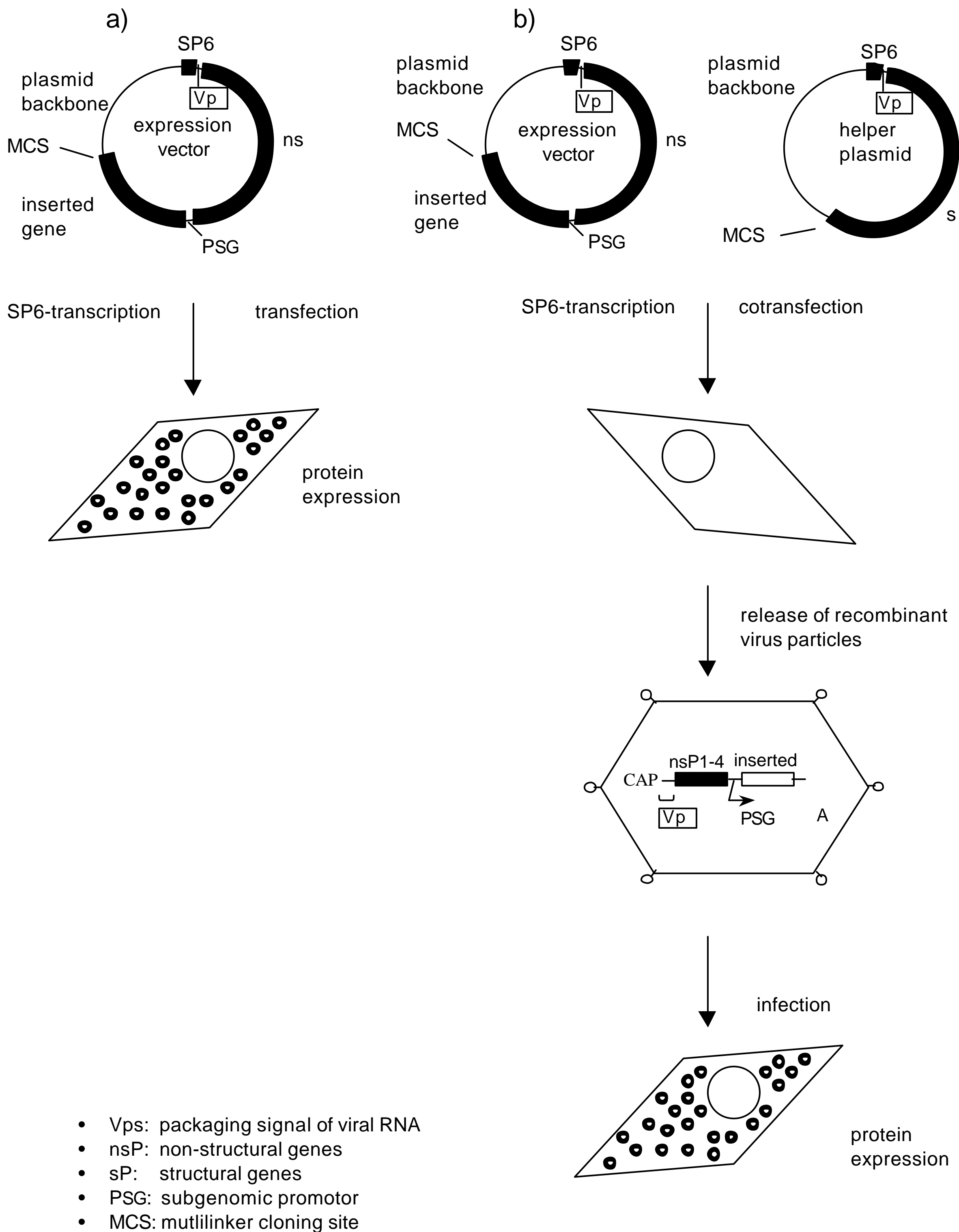
Helper plasmids derived from Semliki forest virus are called pSFV-Helper1 and pSFV-Helper2, helper plasmids derived from Sindbis virus are called DH-BB. The helper plasmids lack the regions coding for the viral non-structural proteins and for the signal for the packaging of the viral RNA, but they contain cDNA coding for the structural proteins under control of the bacteriophage SP6-promotor.

Classification of work with Semliki forest virus/Sindbis virus expression vectors

Vector/activity	group/class
• Expression vector or helper vector in E. coli K12	1
• Transfection of established eukaryotic cells with transcripts from SFV or Sindbis virus expression vectors (Fig. 2a)	1
• Transfection of primary human cells with transcripts from SFV or Sindbis virus expression vectors	2
• Cotransfection of cells with transcript of expression vector containing subgenomic viral or cellular sequences and transcript of helper (Fig. 2b)	2
• Resulting viral particles from this cotransfection	2
• Infection of eukaryotic, including primary human cells	2

Note: In cotransfections of transcripts from pSFV expression vectors and **pSFV helper 2 plasmid** no infectious virus can arise by recombination due to point mutations. In this case the resulting viral particles can be classified in group 1.

Semliki forest virus (SFV) and Sindbis virus expression



Adapted from ZKBS, 1996

Figure 2

Adenovirus vectors

Adenovirus vectors used for gene transfer usually are replication defective due to a deletion in the E1 region. Many vectors also have a deletion in the early region E3 which is not essential for virus replication; some new vectors also have a deletion in early region E4, leading to a defect in late protein synthesis, but not to replication incompetence. In order to produce infectious particles, plasmids containing the defective adenovirus genome and the gene to be expressed are introduced into cells constitutively expressing the E1A genes, such as the human 293 cells.

Work with adenovirus vectors has so far generally been classified in class 2. However, if only individual steps are applied, it is possible to classify some of the steps into class 1, as has been proposed by the German *Zentrale Kommission für biologische Sicherheit* (ZKBS) and as shown below.

Classification of work with adenovirus vectors

Vector/activity	group/class
• Replication-competent adenovirus	2
• Replication-defective, recombinant adenovirus (with or without insert)	2
• Infection of complementing cells with replication-defective, recombinant adenovirus	2
• Infection of non-complementing cells with replication-defective, recombinant adenovirus	2
- If the cells can not complement the replication defect and following demonstration that no virus is shed	1
• Infection of animals which cannot complement the replication defect with replication-defective, recombinant adenovirus	2
- As soon as no shedding can be detected	1
• Free or in pBR-based plasmid inserted intact adenovirus genome	2
• Transfection of non-complementing cells with free or in pBR-based plasmid inserted intact adenovirus genome	2
• Free or in pBR-based plasmid inserted replication-defective adenovirus genome	1
• Transfection of complementing cells with free or in pBR-based plasmid inserted replication-defective adenovirus genome	2
• Transfection of non-complementing E. coli K12 cells with a free or in a pBR-based plasmid inserted replication-defective adenovirus genome [a)]	1

[a)] class 2, if the possibility of complementation exists

adapted from ZKBS, 2000